11th EUROCAT European Symposium
Antwerp, Belgium, 17 June 2011

Attachment: Abstract Book

Ways to Improve Prenatal Diagnosis in the 21st Century

Jacquemyn Y

The development of technology as led to major advances in prenatal diagnosis, but the most recent developments mainly in ultrasound have led to minor, let's call it asymptotic, improvements that due to the high cost of these machines can only be of benefit to the happy few. One ought always bear in mind that the widespread use of less sophisticated techniques and machines is of profit to much more women and their babies than high end machines. In this text I will limit myself to congenital malformations, structural, genetic or functional. Our knowledge on functional malformations or the functional impact of structural malformations is minimal from a prenatal point of view. I will not discuss prediction and prevention if intrauterine growth restriction, preeclampsia and preterm delivery and will not comment on multiple pregnancy, I will also only provide a glimpse on prenatal therapy.

What is the ideal world for prenatal diagnosis? One should aim at 100% antenatal detection, this means that we should not just offer screening towards patients but that we should offer diagnostic modalities to all patient from the beginning. We aim to make diagnosis as early as possible in pregnancy, even before conception. Ideally preconception diagnosis in vivo should be reached. This would allow to treat the fetus as a patient antenatally, offer optimal perinatal support and avoid termination of pregnancy. One can wonder whether he perfect baby is our "holy grail", if not even more " the perfect family". Or is this our nightmare?

Different trends can be discerned in today's prenatal diagnosis. These trends are:
- Diagnosis goes from late in pregnancy to early as illustrated from second trimester amniocentesis and ultrasound to first trimester ultrasound and chorion villi biopsy to preimplantation genetic diagnosis?
- Focus more and more on preconception care.
- Prenatal diagnosis moves from invasive to less invasive to non invasive such as illustrated by congenital hemoglobinopathy which first was diagnosed by cordocentesis, afterwards to polymerase chain reaction on DNA in chorion volli or amniocytes and perhaps diagnosis in maternal blood later on.
- A move from screening and risk determination to immediate diagnosis
- From selective examinations in high risk cases to offering as much test as possible to the general population.

Prenatal diagnosis concerns basically imaging and lab testing. Of course both should be integrated.

On the side of imaging one may expect 3 and 4 dimensional ultrasound to deliver ever increasing image quality. But increasing image quality does not mean that the quality of the operators will increase and the clear image that leaves less to our imagination does not necessarily mean that diagnostic capacity is improved is better. We still wait for convincing evidence of superiority of 4D ultrasound, except in a few fields such as the diagnosis and prognosis of facial abnormalities. I do not expect ultrafast magnetic resonance imaging to really add anything in the future, neither do I think that fetoscopy and embryoscopy will offer new possibilities, certainly not for the general population and in the diagnostic setting. Today we see that prenatal ultrasound has moved to the 11 to 13 weeks scan. For some anomalies a 100% detection rate has been described, including acrania or exomphalos. For others just a 50% detection rate is noted, including diaphragmatic hernia or lethal skeletal dysplasia, for major cardiac anomalies 34% detection rate is noted and for such a frequent and obvious malformation as spina bifida the 11 to 13 week ultrasound scan detection rate is only 14% in highly skilled hands. Perhaps by optimal and repetitive training these numbers can be achieved in all of Europe but even after second trimester scanning todays numbers inEuropemust make us realize most humbly that only 96% of cases of anencephaly, 83% of cases of gastroschisis and 68% of cases of spina bifida are diagnosed before birth. The problem of early detection of spina bifida is intriguing. Probably only the most severe cases and those associated with other major abnormalities are diagnosed in the first trimester. This can be due to the poor ossification of the fetal spine, the small size of the spine and the maternal habitus in our even increasing epidemic of obesity. Recently detailed examination of the posterior fossa at 11-13 weeks scan looking for called intracranial translucency might improve our detection rate. This has not yet been convincingly demonstrated.

In the years to come the list of new markers of aneuploidy will become ever increasing. After nuchal translucency, the nasal bone, the increased impedance in the ductus venosus, tricuspid regurgitation, facial angle, liver volume and more recently increased hepatic artery blood flow have been added. Probably other markers will be added but they will not change much in the real uptake of aneuploidy. Humbly we must realize that there will always be anomalies that can never be detected such as microcephaly at the 11-13 weeks as this develops only after 30 weeks, agenesis of the corpus callosum before 18 weeks because the corpus callosum is not developed until that age, or postinfectious problems that only appear a few weeks after the infectious insult. Most cases of polycystic kidney disease will escape from our early prenatal diagnostic capacities as these disease only develops later in pregnancy. Some other prenatal problems must be always detectable such as anencephaly and some will be detectable sometimes including spina bifida, facial cleft ore multicystic kidney at 11-13 weeks scan.

What can we improve in today's ultrasound?
The detection rate of heart and skeleton anomalies lags far behind the detection rate for other systems. Probably the routine assessment of complete cardiac outflow in all patients, optimizing training of prenatal ultrasonographists and perhaps telecardiology with STIC (spatiotemporal image correlation) volumes analysed by an expert elsewhere will improve this part of prenatal diagnosis.

In the near future optimalisation of prenatal ultrasound can increase the amount of first trimester diagnosis of structural anomalies. This will need appropriate training of the sonographers, we really have to take more time for the scan and include detailed examination of the fetal heart. Every fetus with an increased nuchaltranslucency should be reviewed by an expert in echocardiography. From the technical point the resolution will still become better. 4D-ultrasound will become ever faster and perhaps high intensity focused ultrasound (HIFU) will allow local necrosis of tumors or the external treatment of twin to twin transfusion syndrome, local delivery of drugs or DNA in microspheres. We will also see ever involving miniaturisation of the devices.

For the lab tests in prenatal diagnosis we can look at maternal serum screening versus fetal material in the maternal circulation.
Actually maternal serum screening is basically screening for Down syndrome (DS). The detection rate is up to 96% for false positive rates between 3 and 5%. Looking at trisomy 18 and 13 the detection rate is only 75% with DS algorithms but if one uses a specific algorithm the detection rate increases to 95% with a false positive rate of only 0.1%. Incidentally other abnormalities can be found due to maternal serum screening, including Triple X, Smith-Lemli-Opitz syndrome or X linked ichtyosis.

For fetal material in maternal circulation we mention fetal cells and cell free fetal DNA or RNA. Fetal cells have been “the method of the future” ever since the eighties and actually it is not to be expected that there will be any news from this front. The fast evolution in genetics will influence our possibilities in prenatal diagnosis. As array-based comparative genomic hybridization will be used by more and more laboratories, single nucleotide polymorphism detection will become available in more and more centers and even DNA sequencing including shot gun or massive sequencing will become daily practice.

This will allow single cell analysis and perhaps we will have to widen our horizon not only to the human genome project but also to the human epigenome project. 5% of the DNA in the maternal circulation is fetal. This is already used almost in routine practice for fetal sexing or fetal Rhesus D determination and is emerging as a method to detect aneuploidy as has been demonstrated for Trisomy 18, it can be used for the detection of the paternal genome for dominant disorder such as Huntington and perhaps in the future complete genome analysis will be possible, notwithstanding the ethic and social consequences of knowing your child’s complete genome before birth. Anyhow cell free fetal DNA will allow to determine aneuploidy and a bunch of genetic diseases, thus systematically offering diagnosis instead off screening to all.

Is there still a future for invasive diagnosis?
Probably yes, microarrays on chorion villi provide incredibly more fetal genetic information than classic karyotype and until now still much more than can be expected from the cell free fetal DNA.. It is to expected that in 1% of sample, when performed in a general population will demonstrate significant genetic disease. This results in major problems concerning counseling but it also changes the risk/benefit ratio for the procedure, if at this moment we provide invasive diagnosis for a risk of 1/270 to 1/300 for Down syndrome and balance this with the risk for procedure related fetal loss between 1/500 and 1/100, then of course finding a significant genetic anomaly in 1%, rationally leads to invasive invasive testing for all.

Some other problems will be encountered in the near future because if we go for more screening more “collateral damage” including incidental findings that are difficult to interpret. This results in more anxiety changing prenatal “care” to prenatal “scare”. The obesity epidemic might neutralize the high image resolution development of high definition ultrasound. Where do we stand when we can make very complex diagnoses but are unable to counsel on the functional results of even straightforward diagnoses as hydrocephaly diagnosed in the unborn baby? How to accurately interpret a CMV infection, we are very well capable of demonstrating CMV in the amniotic fluid to prove that the baby is infected but with all our technology we are not capable of telling the parents the functional outcome of their baby. The same kind of uncertainty will result from DNA sequencing. What to tell in case of an incidental finding? What to tell if we know that this fetus is programmed to be autistic, if ever such a diagnosis would be possible?

We can expect the almost classic comments from adversaries of prenatal diagnosis.

It will be argued that prenatal diagnosis can be used for eugenic and social purposes such as sex selection, as is the case inIndia. In Europe this is forbidden by law, not so in theUnited States. But this seemingly "ethic" argument is in conflict with the generally accepted view that every generation should aim for the next generation to have a better live. Such selections are perhaps against classic Western European ethics but are our ethics superior and fit for the future? Others will argue that we strive to eradicate certain diseases. Indeed we are! We want to eradicate diseases, but not individuals. Nobody wants to eradicate happy people with Down syndrome. But we would like to eradicate people being born with cystic fibrosis or thalassemia or hemophylia. What is wrong with this? Everyone agrees that we strive to eradicate tuberculosis and malaria, polio or any other infectious diseases. Who is against the eradication of cancer?

In conclusion I state that the most nearby future development should be the improvement of the detection rate for cardiac and skeleton anomalies on ultrasound and moving from the second to the third trimester for the detection of structural anomalies. Probably cell free fetal DNA in the maternal circulation will replace serum screening for chromosome abnormalities resulting in diagnostic instead of screening tests.

The problem stays that we must wonder how we can make all this high tech care available for all and in the same time still respect the free choice of all to use or not to use these tests.

References

This is not a classic article nor a classic list of references, I just present in alphabetical order a series of recent publications, mainly in Prenatal Diagnosis that have been inspiring to me.

Chitty LS, Lau TK First trimester screening new directions for antenatal care . Prenat Diagn 2011;31: 1-2
Chitty LS. The evolution of prenatal diagnosis. Prenat Diagn 2011;31:599-560
Dennis Lo YM. Noninvasive prenatal diagnosis in 2020. Prenat Diagn 2011;31: 702-703
Lee C. The future of prenatal cytogenetic diagnostics: a personal perspective. Prenat Diagn 2011;31:706-709
Nicolaides KH. Screening for fetal aneuploidies at 11 to 13 weeks. Prenat Diagn 2011;31:7-15
Panigrahi I, Thakur S, Murthy S. Non invasive prenatal diagnosis: improved detection rates. Prenat Diagn 2011;31:221
Pergament E. By 2020… Prenat Diagn 2011;31: 710-711
Robson S. Fetal ultrasound screening and diagnosis 10 years hence. Prenat Diagn 2011;31:696-698
Sepulveda W, Wong AE,FauchonDE. Fetal spinal anomalies in a first trimester sonographic screening program for aneuploidy. Prenat Diagn 2011;31: 107-114
Syngelaki A, Chelemen T, Dagklis Th, Allan L, Nicolaides KH. Cahllenges in the diagnosis of fetal non chromosomal abnormalities at 11-+13 weeks. Prenat Diagn 2011;31: 90-102
The Danish council of Ethics The future of prenatal diagnosis
Wapner RJ. What can we expect from invasive procedures in 10 years time. Prenat Diagn 2011;31:704-705

Preconceptional Care to Improve Pregnancy Outcome

Steegers E

Despite major advances in medical care and the relatively high level of prosperity, poor perinatal outcomes continue to be a major problem in highly developed countries such as theNetherlands[1]. This has stimulated a paradigm shift in strategies to improve women's health and pregnancy outcomes from a focus on the prenatal period to the preconceptional period [2]. This type of care is called Preconception care (PCC). PCC is a set of interventions that aim to identify and modify biomedical, behavioural, and social risks to a woman's health or pregnancy outcome through prevention and management [3]. There is sufficient evidence for several components of PCC and therefore this new care should be consistently offered to

women in their reproductive years [4-7]. Efforts have to be put in intervention methods and strategies to provide effective PCC. In this regard the following aspects need to be taken in to account: 1) a distinction in general and specialised PCC. It must be clear which components of PCC are provided by general practitioners and midwifes and which components are provided by medical specialists like obstetricians and clinical geneticists; 2) protocols should be drawn up to enable to provide care in a uniform manner; 3) professionals providing PCC need to be trained; 4) risk assessment tools need to be developed and standardised; 5) communication tools and strategies need to be developed and adapted to the local situation; 6) municipal public health institutions and schools need to be involved.

In the Netherlands, several pilot studies on *general* individual preconception care provided by midwives, obstetricians and clinical geneticists have been organized. A self-administered Internet questionnaire for preconception risk assessment for the general population (www.zwangerwijzer.nl) was developed in 2004, subsequently validated [8,] and appears to be successful. Such information on risk factors can be used by the caregiver in providing preconception care. Depending on the risk factors, the couple may be referred for *specialist* individual preconception care. Regional and national protocols are being developed to guide such chain system of care in low- and high risk populations. A web based instrument (www.preconceptiewijzer.nl), designed for the caregiver, is now being tested, coupling risk factors with protocols for preconception advice.

In 2009 the municipal councilRotterdamand the Erasmus University Medical Centre started a city wide urban perinatal health program to improve perinatal health outcomes [9]. Preconception care is a key element of this ten-years program. Highest priority is given to reach out to ethnic minorities and couples with a low socioeconomic status.

References

[1] Mohangoo AD, Buitendijk SE, Hukkelhoven CWPM, Ravelli ACJ, Rijninks-van Driel GC, Tamminga P en Nijhuis JG. Hoge perinatale sterfte in Nederland vergeleken met andere Europese landen: de Peristat-II-studie. Ned Tijdschr Geneeskd. 2008;152:2718-27 (https://www.ntvg.nl/publicatie/hoge-perinatale-sterfte-nederland-vergeleken-met-andere-europese-landen-de-peristat-ii-st/volledig).
[2] Moos MK. Preconceptional health promotion: progress in changing a prevention paradigm. J Perinat Neonatal Nurs. 2004;18:2-13.
[3] Berghella V, Buchanan E, Pereira L, Baxter JK. Preconception care. Obstet Gynecol Surv. 2010;65:119-31.
[4] Jack BW, Atrash H, Coonrod DV, Moos MK, O'Donnell J, Johnson K. The clinical content of preconception care:an overview and preparation of this supplement. Am J Obstet Gynecol. 2008;199(6 Suppl 2):S266-79.
[5] Hillemeier MM, Downs DS, Feinberg ME, Weisman CS, Chuang CH, Parrott R, Velott D, et al. Improving women's preconceptional health: findings from a randomized trial of the Strong Healthy Women intervention in the Central Pennsylvania women's health study. Women's Health Issues. 2008;18(6 Suppl):87-96.
[6] Whitworth M, Dowswell T. Routine pre-pregnancy health promotion for improving pregnancy outcomes. Cochrane Database Syst Rev. 2009 Oct 7;(4):CD 007536.
[7] Johnson K, Posner SF, Biermann J, Cordero JF, Atrash HK, Parker CS, Boulet S, et al. Recommendations to improve preconception health and health care-United States. A report of the CDC/ATSDR Preconception Care Work Group and the Select Panel on Preonception Care. MMWR Recomm Rep. 2006;55:1-23.
[8] Landkroon AP, de Weerd S, van Vliet-Lachotzki E, Steegers EA: Validation of an internet questionnaire for risk assessment in preconception care. Public Health Genomics 2010; 13:89-94.
[9] Denktas S, Voorham T, Bonsel G, Spittje J, Huizer M, Willems M, de Graaf J, van der Weg E, Peters I, Steegers EA: Grootstedelijke perinatale gezondheid. Programmatische aanpak van perinatale sterfte in Rotterdam. Tijdschrift voor Sociale Geneeskunde 2009; 87:199-202.

Preconceptio Care for Maternal Diabetes Prevents Congenital Anomalies

Hawthorne G

Background

Molsted –Pedersen described the high incidence of congenital malformation in offspring of women with diabetes in1964. In1994, I was a funding member of the Northern Diabetic Pregnancy Survey and we audited diabetic pregnancy outcomes in the Northern Region of England. In our diabetic population the congenital malformation rate was 83/1000 compared to 21.3/1000 for the background population. Six percent of all fetal losses were due to fetal malformation and 2 late neonatal deaths were due to congenital heart malformations [1].

Since then other studies have confirmed the three fold increased risk of congenital anomaly in offspring of diabetic mothers and CEMACH in 2002/03 demonstrated that inEngland,WalesandNorthern Irelandthe prevalence of congenital anomaly for offspring of women with diabetes was 48/1000 for Type 1 diabetes and 43/1000 for Type 2 diabetes. There was a 4.2 fold increase in neural tube defects and a 3.4 fold increase in congenital heart disease [2].

Pathogenesis

Congenital malformations occur in the first 6-7 weeks of gestation. Caudal regression occurs at 3 weeks post fertilisation, spina bifida and anencephaly at 4 weeks and transposition and renal abnormalities at 5 weeks. Our data show that maternal diabetes is associated with a fivefold increase in cardiovascular malformation and that transposition of the great vessels [17 fold excess], truncus arteriosus and tricuspid atresia are over represented.[3]

Hyperglycaemia during critical periods of morphogenesis is regarded as the major teratogen in diabetic pregnancies although the causative molecular mechanisms are unknown. In the mouse diabetes model there is evidence that maternal diabetes alters expression of developmental genes in the embryo. It is hypothesised that diabetic pregnancy leads to altered expression of molecules that play key roles in patterning and development of embryonic tissue implicating altered transcriptional regulation as a possible pathogenic mechanism. Currently a unique European wide collaboration, the Congenital heart and environment/epidemiological database [CHEART], is focussing on the mechanisms of normal and abnormal heart development leading to congenital heart disease. This study is testing whether genetic variants are associated with cardiovascular malformation and is investigating gene-environment interactions with maternal diabetes using a genome wide association study.

Evidence to support preconception care

Preconception care was first demonstrated to be effective by Kurt Fuhrmann in 1973 [4]. He showed a reduction of congenital malformation from 5.5% to 0.8% by the introduction of tight glucose control prior to conception. Several studies have replicated this finding and a meta-analysis of 14 studies of the effect of preconception care showed that lack of preconception care is associated with a 3 fold increase in the risk of major and minor malformations [5].

Implementation of preconception care

What is preconception care? NICE [6] recommends that avoidance of unplanned pregnancies is an essential component of diabetes education for all women of child bearing age and that:

- Pregnancies should be planned and women should be fully informed of the risks associated with diabetic pregnancy.
- Contraception should be continued until good glycaemic control as measured by HbA1c is established.
- All women should take folic acid 5mg/day from preconception till 12 weeks gestation.

So if the evidence is compelling why are all diabetic women not getting preconception care? A survey by CEMACH showed that inEngland,WalesandNorthern Irelandonly 17% of units had a preconception clinic [7]. Widespread implementation of the delivery of preconception care has been patchy with variable success.

Recently the NHS inEnglandhas developed a web based information strategy to support commissioners and providers of care and women with diabetes. This national strategy to improve the uptake and delivery of preconception care was launched in March 2011.It remains to be seen how effective this will be.

References

1. Prospective population based survey of outcome of pregnancy in diabetic women: results of the Northern Diabetic Pregnancy Audit,1994. GHawthorne,S Robson, EA Ryall, D Sen, SH Roberts, MP Ward Platt. BMJ 1997 315:279-281.
2. Macintosh MCM, Fleming KM, Bailey JA, Doyle P, Modder J, Acolet D, Golightly S, Miller A. Perinatal mortality and congenital anomalies in babies in women with type 1 or type 2 diabetes inEngland,WalesandNorthern Ireland: a population based study. BMJ 2006 333:177
3. Wren C, Birrell G, Hawthorne G. Cardiovascular malformations in infants of diabetic mothers. Heart 2003:89:1217-1220
4. Fuhrmann K, Reiher H, Semmler, Fischer F, Fischer M, Glockner E. Prevention of congenital malformations in infants of insulin- dependent diabetic mothers. Diabetes Care 1983 6:219-223
5. Ray JG, O'Brien TE, Chan WS. Preconception care and the risk of congenital anomalies in the offspring of women with diabetes mellitus: a meta-analysis. Q.J Med 2001 94:435-444
6. NICE guideline 63:Diabetes in pregnancy. Management of diabetes and its complications in pregnancy from the pre-conception to the postnatal period 2008 www.nice.org
7. Confidential enquiry into maternal and child health. Maternity services in 2002 for women with type 1 and type 2 diabetes. CEMACH 2004.

Preconceptional or Prenatal Fragile X Syndrome Screening

Hilbert P

Fragile X syndrome is one of the most frequent cause of inherited mental retardation. The high frequency of this disease might justify the prenatal or preconceptional screening of women but the question remains controversial. We present here the results of our 19 years experience in this analysis.

Prenatal or preconceptional screening was performed on request of clinicians, mainly geneticists and gynaecologists, for women with no known family history of fragile X syndrome. On a total of 14654 women, a full mutation (more than 200 repeats) was found in one patient and a premutation (55-199 repeats) in 78 patients (1/185).

Genetic counselling was recommended to all premutated women : results were explained to the patients, familial screening was performed and enables the identification of other premutation carriers and availability of prenatal diagnosis was discussed. On a total of 52 foetus from 39 different women, the premutated allele was transmitted 24 times. In 19 cases, mothers had expansion below 70 repeats and the inherited premutation in the foetus ranged from –3 to +12 repeats compared to the mother's premutation. In 5 cases, the mother's expansion was over 70 repeats. In 4 males, the transmission resulted in 3 full mutations and 1 mosaïcism for full mutation and premutation and , in one female, in an unmethylated expansion of about 200 repeats. Pregnancy was terminated in the 4 affected males. This confirms that alleles over 70 repeats are at high risk of expansion to full mutations when transmitted. Such alleles concerned 21 of the 78 premutated women which means 1/697 women of an unselected population.

The debate on the eventuality of wide-scale fragile X syndrome screening in childbearing age women is still tricky. Some publications show interesting data at epidemiological, ethical, or economical points of view. In addition with those different studies, our results could be useful to discuss the interest of such a screening.

Human Biomonitoring: Prenatal Exposure and Early Effects on the Newborn
Schoeters G

Animal toxicology studies, human case studies and occupational exposures have documented that exposure to chemicals at high doses may cause toxic effects on the foetus and newborns. It is much more difficult to predict health effects of continuous exposure to low doses of chemicals such as from air pollution, contaminants in food, chemicals in consumer products (1). Human biomonitoring measures concentrations of chemicals and early effects in human tissues and fluids. Its use in population studies has increased the weight of evidence for linking specific environmental exposures to adverse health effects. Some of the effects may be subtle at the individual level, but may become significant at the population level. Exposure of the developing organism is recognized as a particular concern.
Environmental chemicals which are present in the maternal body may reach the developing embryo and foetus. The mother's body acts as a reservoir. Chemicals selectively pass the placenta. To obtain information on early life exposure, chemical concentrations can be measured in different periods: prenatally in maternal tissue or fluids, at birth in cord blood or placenta, after birth in maternal milk. Human biomonitoring of the general population allows to evaluate time trends in exposure to environmental chemicals and allows to identify high exposure groups among the population. The choice for analysis of eg. blood samples or urine samples depends on the chemicals under study. Exposure to persistent lipophilic chemicals (polyhalogenated compounds such as PCBs, HCB, DDE and PBDEs) are preferentially measured in plasma lipids, while non persistent chemicals such as organophosporous pesticides are preferentially measured through urinary chemical/metabolite concentrations (2). Non-invasive sampling methods such as maternal hair, nails, teeth, expired air are under development and may offer interesting opportunities for biomonitoring of the general population

A range of endpoints have been associated with prenatal chemical exposures. The brain seems particularly sensitive to toxic exposures. During brain development, a complex series of steps must be completed in the right sequence and at the right time. Slight decrements in brain function may have serious implications for future social functioning and economic activities, even in the absence of mental retardation or obvious disease. There were several benchmarking studies which have shown that increased prenatal exposure to methylmercury and PCBs in various cohorts with high fish consumption are associated with delay in cognitive, behavioral, sensory and auditory development. In the Faroe Islands birth cohort cord blood mercury levels were unfavorably associated at age 7 years with test scores for attention, language and memory and at ages 14 for verbal and attention scores (3). In a Dutch cohort, cord plasma and breast milk noncoplanar PCB congener levels have also been associated with cognitive deficits in children age 6–7 years. Also susceptibility to infections, effects on onset of asthma and allergies, effects on growth and puberty development have been associated with chemical exposures. Perinatal exposure to endocrine-disrupting chemicals, such as polychlorinated or polybrominated biphenyls or dichlorodiphenyltrichloroethane compounds have shown to affect puberty development and sexual maturation at adolescence, although not all studies are conclusive and sometimes conflicting results are obtained (4).

Recent concerns about globally observed trends in shortened gestational age and decreased birth weight and the related disease burden have introduced the hypotheses that specific chemicals may alter metabolic programming during prenatal exposure. Fetal growth retardation and low birth weight outcome have been recently associated with prenatal biomarkers of chemical exposures. Several studies reported clinically significant decreases in birth weight of 100 to200 gif lower and higher exposure percentiles are compared. The magnitude of effect is considerably equal or more than that reported for cigarette smoking (~ 55–189 g reduction). Few studies report on post natal increases in BMI in relation to prenatal exposure. 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p' DDE) has been associated with increased BMI.

Biomarkers of exposure can be complemented by analysis of effect biomarkers and susceptibility biomarkers. The effect biomarkers reflect early biological changes in line with the mode of action of the chemicals such as genotoxicity, endocrine disruption, immune changes, oxidative stress. Recently "omics"

technology has been introduced in biomonitoring of the general population allowing high throughput analysis of changes in gene expression, or changes in urinary metabolite or protein profiles. This holds promises for elucidating toxicological pathways through which chemicals cause adverse health effects.

Circulating hormone levels in cord blood have been used as biomarkers for prenatal exposure to environmental chemicals which may act upon hormonal pathways. Hormones, their transporters, their receptors on target organs, and their metabolic pathways are possible targets for endocrine disruptors. Several studies have looked at interference of environmental chemicals with the thyroid system and with circulating sex hormones (5). **DNA adducts** have been measured in placenta and in cord blood lymphocytes in response to air pollution. It is well known that particulate matter and associated metals and polyaromatic hydrocarbons may cause oxidative damage resulting in DNA adducts and in a changed *in utero* environment (6). Changes in the relative **distribution of lymphocyte immunophenotypes** in cord blood is another effect marker which has been associated with prenatal polyaromatic hydrocarbon (PAH) pollution and may be one of the first signals of the early impact of environmental exposure on the immune pathways (7) Recently an exploratory study identified an epigenetic marker in human umbilical cord white blood cells which was associated with transplacental PAH exposure and with the risk of developing asthma symptoms prior to age 5. Epigenetic changes may provide a mode of action for prenatal induced changes which may become manifest later in life. Recent molecular epidemiological studies have identified a number of environmental toxicants that cause altered methylation of human repetitive elements or genes.

To conclude: the mother's chemical body burden will be shared with her fetus or neonate, and the child may, in some instances, be exposed to larger doses relative to the body weight. Developmental exposures to environmental chemicals can lead to a wide range of functional deficits which become manifest after birth and may have life- long health impacts. To understand the underlying mechanisms, molecular markers should be introduced in long-term prospective studies, and existing and planned pregnancy or birth cohorts.

Acknowledgement:

EU-COPHES ref.244237 European coordination action on human biomonitoring, **CO**nsortium to **P**erform **H**uman biomonitoring on a **E**uropean **S**cale), **EU-OBELIX** contract: FOOD- ref.227391 Obesogenic endocrine disrupting chemicals: linking prenatal exposure to the development of obesity later in life, Expertise Centre of Environmental health (Ministry of the Flemish Community, Department of Science, Department of Public Health and Department of Environment).

1. Wigle DT, Arbuckle TE, Turner MC, Bérubé A, Yang Q, Liu S, Krewski D.Epidemiologic evidence of relationships between reproductive and child healthoutcomes and environmental chemical contaminants. J Toxicol Environ Health B Crit.Rev. 2008; 11(5-6):373-517.
2. NeedhamLL, Calafat AM, Barr DB. Assessing developmental toxicant exposures via biomonitoring. Basic Clin Pharmacol Toxicol. 2008;102(2):100-8.
3. Grandjean P, Landrigan PJ. Developmental neurotoxicity of industrial chemicals. Lancet. 2006; 16;368(9553):2167-78.
4. Schoeters, G., Den, H. E., Dhooge, W., Van, L. N., & Leijs, M. 2008. Endocrine disruptors and abnormalities of pubertal development. ***Basic Clin Pharmacol Toxicol***, 102(2): 168-175.
5. Maervoet J, Vermeir G, Covaci A, Van Larebeke N, Koppen G, Schoeters G, Nelen V, Baeyens W, Schepens P, Viaene M. Association of thyroid hormone concentrations with levels of organochlorine compounds in cord blood of neonates. Environmental Health Perspectives 2007;115 : 1780-6.
6. Srám RJ, Binková B, Dejmek J, Bobak M. Ambient air pollution and pregnancy outcomes: a review of the literature. Environ Health Perspect. 2005;113:375-82.
7. Hertz-Picciotto I, Herr CE, Yap PS, Dostál M, Shumway RH, Ashwood P, Lipsett M, Joad JP, Pinkerton KE, Srám RJ. Air pollution and lymphocyte phenotypeproportions in cord blood. Environ Health Perspect. 2005;113(10):1391-8.
8. Perera F, Tang WY, Herbstman J, Tang D, Levin L, Miller R, Ho SM. Relation of DNA methylation of 5'-CpG island of ACSL3 to transplacental exposure to airborne polycyclic aromatic hydrocarbons and childhood asthma. PLoS One. 2009;4(2):e4488.

Swine Flu Vaccination During Pregnancy, Follow-Up Data

Schaefer C

Pregnant women may be at high risk for severe complications as known from some previous influenza pandemics and seasonal influenza resulting in a higher rate of hospital and intensive care unit admission and death. Beside morbidity and mortality, adverse pregnancy outcomes have been reported following previous influenza pandemics, e.g. birth defects, spontaneous abortion, fetal death, and preterm delivery. In 2009, mass vaccination campaigns against the pandemic H1N1v strain had begun in many countries with pregnant women as a priority group, although the safety of the novel vaccines in pregnancy could not be assessed due to the short time of the product development and the fact that pregnant women are excluded from clinical trials.

The Berlin Teratology Information Service (TIS) was commissioned by the Federal Ministry of Health to counsel physicians and pregnant women about influenza A (H1N1)v 2009 vaccination and to register and evaluate pregnancy outcomes. On September 28th, 2009, the surveillance has been started. Subjects have been prospectively enrolled using a structured questionnaire at the first contact during pregnancy. The following information was obtained: brand name of the vaccine, batch number, reported local or systemic adverse effects after vaccination, timing in pregnancy, details on concomitant medication, flu like symptoms, fever, infections and chronic diseases, maternal demographics, and obstetric history. A comparison group (n=1.636) was recruited during the same time period, i.e. matched for the estimated date of birth consisting of pregnant women who had been counseled during pregnancy about exposures known to be non-teratogenic. Until March 31, 2010, 359 pregnant women were enrolled who met the criteria for the study, i.e. resident in Germany and exposed to influenza A (H1N1)v 2009 vaccine during pregnancy or less than four weeks prior to conception. A follow-up could be initiated in 344/359 cases. By March 15, 2011 follow-up was completed for 317 (92%) pregnancies. 217 of these women were vaccinated with the non-adjuvanted split vaccine CSL H1N1 Pandemic Influenza Vaccine® (CSL Biotherapies), which was exclusively introduced for pregnant women in January, 2010. In contrast, the AS03-adjuvanted split vaccine Pandemrix® (GlaxoSmithKline) was documented in 86/317 cases, what might reflect the uncertainty and public discussions regarding the use of adjuvanted vaccines in pregnancy. Two patients were vaccinated with the MF59-adjuvanted split vaccine Focetria® (Novartis) and for 12 patients product information could not be obtained.

Based on the preliminary evaluation of our data, swine flu vaccination does not seem to be associated with adverse pregnancy outcomes (table 1). So far, the rate of major birth defects, the pattern of anomalies and the frequency of other outcomes including preeclampsia do not indicate substantial teratogenicity or developmental toxicity of the vaccine. However, since the cohort under study is still rather small and most of the patients were vaccinated after the first trimester we cannot rule out a risk. A final conclusion will be drawn after completion of all enrolled cases, comparison with the control group, and statistical analysis of the data.

Table 1: Pregnancy outcome after (H1N1)v 2009 vaccination

Pregnancy outcome	Number of cases
FUP completed	317
spontaneous abortion	6 / 317 (including 1 anencephaly)
elective termination of pregnancy	3 / 317 (including 1 trisomy 21)
live births	308 / 317 (312 live born including 4 pairs of twins)
all congenital anomalies *	28 / 314 (8.9%)
major birth defects *	8 / 313 (2.6%)
preeclampsia	10 / 317

*Number of anomalies by the number of live-born children (n=312) plus pregnancy losses with malformations (n=2). For calculation of major birth defects chromosomal/genetic abnormalities were excluded.

Investigating Socioeconomic Inequalities, Risk of Congenital Anoamly and Infant and neonatal Mortality
Budd J

Objective: To investigate the effect of socioeconomic inequalities on congenital anomaly occurrence and pregnancy outcome, and assess their impact on the widening deprivation gap in infant and neonatal mortality in theUK.

Methods: Population based study of 646749 births in the East Midlands andSouth Yorkshire1998-2008. Outcome measures were time trends in socioeconomic variation in the risk of anomaly, time of detection, pregnancy outcome and live birth prevalence. Deprivation measured using theUKindex of multiple deprivation 2004.

Results: Non-chromosomal anomaly rates increased significantly with deprivation. Fetuses from the most deprived area had a 54% higher risk compared with the least deprived (95% CI (1.27,1.85). Although antenatal detection rates were similar, rates of termination were lower in the most deprived areas (62%) compared with the least deprived (77%) (RR 0.81 (0.63,1.03)). Consequently, livebirths with a non-chromosomal anomaly were 92% higher (95%CI (1.39,2.67).

Chromosomal anomalies significantly decreased with increasing deprivation (RR 0.54 (0.46,0.63)) due to maternal age (adjusted RR 0.99 (0.85,1.15)). Antenatal detection rates were lower in more deprived areas (RR (0.81 (0.67, 0.97)) with pregnancies less likely to end in termination (RR 0.78 (0.64,0.97)). After adjusting for maternal age, women from the most deprived areas were at 87% increased risk of a livebirth with a chromosomal anomaly (95%CI (1.45,2.40)) .

Conclusions: The increase in non-chromosomal congenital anomalies over time, small decreases in rates of termination and socioeconomic variation in pregnancy outcome has resulted in widening, rather than narrowing, the deprivation gap of live born infants with a congenital anomaly.

Validity and Reliability of Maternal recall of Prescription Drug Use During Pregnancy
Bakker M

In case-control studies that assess associations between drug use and birth defects, detailed information on the type of drug and the timing of use is essential to prevent information bias. However, data on the accuracy of recall of drug use during pregnancy are scarce. Therefore, we validated a self-administered questionnaire by comparing it to pharmacy data which were checked for compliance by maternal interviews. Sensitivity, specificity, levels of agreement, and kappa statistics were calculated to quantify the validity and reliability of the questionnaire for any prescription drug use, groups of drugs, and individual drugs. In addition, we determined whether maternal characteristics influenced the reliability of the questionnaire. A total of 560 women were included. The sensitivity of the questionnaire for any prescription drug use was 57%, ranged between 12% (corticosteroids) and 83% (antihypertensives and thyroid therapy) for drug groups, and between 0% (naproxen) and 73% (salbutamol) for individual drugs. Overall, specificity was high (93–100%). The sensitivity of the questionnaire for prescription drug use during the first 4 months of pregnancy was generally comparable or better than the sensitivity in the complete pregnancy period. Smoking during pregnancy and completing the questionnaire >1 year after delivery decreased maternal recall of prescription drug use. In conclusion, the validity of maternal recall of prescription drug use during pregnancy is generally moderate to poor. As reliability also differed according to some maternal characteristics, future retrospective studies on the teratogenic risk of drug use may need additional sources of data next to self-reported methods.

Grown Ups with Congenital Heart Disease

Suys B

Congenital heart diseases are the most common birth defects in humans, affecting approximately 0,8% of all live births. The spectrum of defects is broad, ranging from complex defects that result in profound disability and death in infancy to minor defects that are discovered sometimes only later in life in asymptomatic adults. Before the era of congenital heart surgery a half century ago, fewer than 30% of children with serious congenital heart defects survived to become adults. Considerable progress in pediatric cardiac surgery since then has led to a remarkable improvement in the life expectancy and quality of life of patients with congenital heart disease. Advances in surgical techniques, catheter interventions, anesthesia, and intensive care have rendered virtually all forms of congenital heart disease (CHD) treatable, and infant mortality from CHD has dramatically decreased. Currently, approximately 85% of children with CHD are surviving to adulthood.
The number of grown-up patients with congenital heart disease (GUCH) is constantly increasing and will equal the number of children requiring surgery for CHD. Patients with congenital heart disease however require lifelong medical care. As a result of the above mentioned improvements, the challenges of caring for GUCH patients are only now being realized. Specialized centers dealing with the medical and paramedical problems of these patients are required. We need to educate patients and families about the need for lifelong and skilled surveillance and care.

GUCH patients can be 'roughly' divided in 6 groups, each with special needs and attention:
1. Patients with minor cardiac malformations presenting at adult age for first treatment
2. Patients presenting for correction as adults because they are either naturally balanced or were surgically palliated
3. Patients presenting for expected reoperations after correction in childhood
4. Patients requiring repair of residual defects after correction
5. Patients developing heart failure after correction or palliation of CHD and possibly requiring transplantation
6. Patients developing acquired heart disease in addition to CHD

The grown-up congenital heart patient is not simply a larger subject compared to the CHD child subject. The cardiac lesion by itself is not always the major problem for these patients, since issues pertaining the quality of life and psychosocial aspects (disability, insurance, work, sports, …) often predominate. It is therefore important to design, adapt and optimize the specific needs.

One of these specific problems is that most women with congenital heart disease are now expected to reach childbearing age, and maternal cardiac disease is the major cause of maternal morbidity and mortality. Appropriate pre-pregnancy counselling and management during pregnancy are fundamental components of the care of these patients.

The care for congenital heart disease is a 'long road'…

References

1. Update in adult congenital cardiac surgery. Daebritz SH. Pediatr Cardiol 2007, 28: 96-104
2. Caring for adults with congenital cardiac disease: successes and challenges for 2007 and beyond. Dearani JA et al. Cardiology in the Young. 2007, 17: 87-96.
3. Best practices in managing transition to adulthood for adolescents with congenital heart disease; the transition process and medical and psychosocial issues. Sable C. et al. Circulation 2011; 123: 1454-1485.

20 years Survival of children Born with Congenital Anomalies

Rankin J

When a child is born with a congenital anomaly, one of the many questions asked by parents of the medical professionals involved in their care is around the long-term health implications for their child. While advances in fetal and neonatal care have improved the prognosis for individuals with certain anomalies such as Down's syndrome and spina bifida, information on long-term survival is still lacking for many important congenital anomalies. Further, the few studies in the published research literature only consider survival to one year of age and for a limited number of conditions. There are also few studies which have examined the influence of a range of fetal and maternal characteristics on survival status.

In this presentation, I will provide an overview of ongoing work on survival and predictors of survival for children born with a range of congenital anomalies. We have used prospectively collected, population-based data on congenital anomalies extracted from the UK Northern Congenital Abnormality Survey (NorCAS) for the study period 1985 - 2003. The NorCAS surveys the geographically defined area of the North of England (UK) which has a population of about three million and approximately 32,000 births per year. NorCAS is notified of cases from multiple sources. All anomalies are coded according to the WHO International Classification of Disease (ICD) version 10 and categorised into congenital anomaly group, subtype and syndrome according to current EUROCAT guidelines. Importantly, congenital anomalies were classified hierarchically to take into account the influence of co-incident anomalies on survival. Data on congenital anomalies were matched to three high quality sources of death information: the Northern Perinatal Mortality Survey, a population based survey of all perinatal and infant deaths in the North of England; death registrations from the UK Office for National Statistics; and local hospital mortality records, to determine the survival status of liveborn children. Cases that could not be traced by any of these methods were excluded from the analysis.

Survival status was available for 99•0% of the total sample. 20-year survival was 85•5% (95% CI 84•8–86•3) in individuals born with at least one congenital anomaly and year of birth was a highly significant predictor of survival. As expected, survival varied between subtypes within the same congenital anomaly group; for example, 20-year survival of individuals born with spina bifida with hydrocephalus was lower than that for individuals with spina bifida without hydrocephalus.

This study provides robust estimates of survival for a range of congenital anomaly subtypes, many of which have not been reported previously. It has also demonstrated the importance of presenting survival data for congenital anomalies by subtype because of the high heterogeneity within congenital anomaly groups. This data provides prognostic information to parents and health professionals, and will help to guide commissioning not only for the future health care needs but also the educational and social care requirements of affected individuals and their families. Further long-term studies are needed from other regions and countries to provide good quality data on survival and to further understand the factors that influence survival.

Therapy Protocol and OUtcome of Schisis Patients in Antwerp

Nadjmi N

Our treatment strategy for cleft lip and palate, which is a multi-step one, is discussed in detail. The timing and the used technique in different treatment steps are of crucial importance to enhance the functional and aesthetic results. The first step in the reconstruction of a complete cleft lip & palate is the lip-adhesion combined with presurgical orthopedic, using a molding plate. This is performed at 3 months of age.

A modified Rotation-Advance technique of lip repair together with a primary correction of the nasal tip deformity is performed 3 months later.

The technique of cleft lip and nose repair is presented in detail, as how the cupids bow is rotated down and the secondary defect is filled. Reconstruction of the white roll and the wet line are discussed. Advancement

of the lateral segment without the incision around the base of the ala (conventional Millard) is our method of choice.
The primary nasal repair is essential. The depressed and displaced alar cartilage must be repositioned in an anatomical position at the time of the primary lip repair.
The mid long term esthetic results are presented and discussed in detail.

At nine month of age a modified Furlow double opposing Z-plasty is performed to reconstruct the soft palate. The width of the hard palate cleft decreases significantly after the closure of the soft palate. We have been reconstructing the hard palate at the age of 18 to 24 months.

A secondary bone grafting is performed at the age of 6 to 9. The timing of maxillary expansion, before or after alveolar bone grafting is discussed.

The secondary correction of the lip and/or nasal tip are usually performed at the age of 5 to 6. Further the timing of orthodontic treatment, transversal and/or sagital distraction osteogenesis, and eventual orthodognathic surgery in cases with growth deficiencies are discussed.

Impact of Maternal Obesity on Neural Tube Defects and Cardiovascular Anomalies
Rankin J
OBJECTIVES: To derive estimates of the absolute and attributable risks of neural tube defects and cardiovascular anomalies for obese women inEngland, and predict changes in prevalence resulting from trends in BMI.

METHODS: The BMI profile of the maternal population ofEnglandand of the prevalence of each outcome were obtained from nationally representative sources. Trends in BMI were modelled by logistic regression. Risk ratios for neural tube defect and cardiovascular anomaly were derived from a recent systematic review. Absolute risks, attributable risks, and future prevalence were estimated.

RESULTS: The estimated absolute risks of a neural tube defect or cardiovascular anomaly for an obese (BMI≥30kg/m2) pregnant woman inEnglandare 19 (95% confidence interval: 1.6-2.2) and 75 (66-84) per 10,000 births respectively, compared to 10 (9.5-11.4) and 60 (67-63) per 10,000 births respectively for women of recommended BMI (25-29kg/m2). An estimated 9.8% (5.6-14.1) of neural tube defects and 3.0% (0.5-5.4) of cardiovascular anomalies inEnglandare attributable to maternal obesity.

If maternal BMI trends continue, 24.0% (22.1-25.9) of the maternal population ofEnglandwill be obese by 2020. This is predicted to result in increases of 6.5% and 4.3% respectively in the prevalences of neural tube defects and cardiovascular anomalies compared with the prevalence in 2010.

CONCLUSION: This study provides estimates of the individual risk and population burden of neural tube defects and cardiovascular anomalies inEnglandresulting from maternal obesity. The results have implications for public health planning and for providing information to obese women about their pregnancy-related risks.

Risk Factors for Anorectal Malformations Using Data of 17 EUROCAT Registries
Wijers C

Objective
The etiology of congenital anorectal malformations (ARM) is still largely unknown. We aimed to identify risk factors for ARM using EUROCAT.

Method
We performed a case-control study based on data from 17 EUROCAT congenital malformations registries between 1980 and 2008. Registers included livebirths, stillbirths and terminations of pregnancy following prenatal diagnosis. Cases were 1,393 children with ARM without a syndrome or genetic defect, of whom 631 were isolated, 629 had one or more other defects, and 133 had the VACTERL (Vertebral, Anal, Cardiac, Tracheo-Esophagus, Renal, and Limb)-association. Controls were 19,876 children with a syndrome or known genetic defect. Preliminary analyses were performed using registered information on maternal age, assisted conception, and multiple pregnancies.

Results
Mean age of case mothers (28.9 years) was lower than that of control mothers (33.1 years). Assisted conception, adjusted for maternal age, increased the risk of having a child with ARM (OR=1.66; 95%CI: 1.21-2.27). No association was found between assisted conception and isolated ARM (OR=1.14; 95%CI: 0.66-1.98), whereas assisted conception increased the risk of ARM with other defects (OR=1.85; 95%CI: 1.20-2.85) and VACTERL (OR=2.88; 95%CI: 1.42-5.84). ARM was also associated with being a twin or triplet (OR=1.76; 95%CI: 1.18-2.63), after adjustment for maternal age and assisted conception. Results were comparable when the analyses were limited to only livebirths.

Conclusion
These preliminary results demonstrate associations between ARM and assisted conception and multiple pregnancy. More thorough and extended analyses may provide additional interesting findings with regard to risk factors for ARM.

Survey of Children with Down's Syndrome
Wojciechowski M

Objective
To describe health problems and future needs of children with Down’s syndrome seen in theAntwerpUniversity Hospital Down team.

Method
Retrospective review of medical records of children seen between 01-01-2008 and 31-03-2011.

Results
111 children, 62 boys and 49 girls, age range 0-20 years, were registered. 97% had a free trisomy 21, 1% a Robertsonian translocation, 2% a mosaic form.
49.5% had a congenital heart defect, 2.7% a congenital gastro-intestinal defect, 1.8% congenital cataract. Furthermore there were multiple acquired problems: thyroid dysfunction in 30.6%, gastrointestinal problems in 10.8%, hearing loss in 45%, vision problems in 43.2%, obstructive sleep apnea in 15.3%, stomatologic problems in 7.2%, epilepsy in 6.3%, overweight in 3.6%, orthopedic problems in 4.5%. Feeding difficulties were found in 45% of the children < 3 years. Need for professional early intervention in developmental, psychological and social issues was identified.

Conclusion
Children with Down’s syndrome have multiple, complicated and specific health problems requiring a multidisciplinary approach. Early recognition, treatment and prevention of medical health problems is an essential contribution to intellectual development, social interaction and as much autonomy as possible. The medical aspects of care are covered by our team but there is a great need for early intervention in other domains to get full benefit of our efforts.

Congenital Anomalies in Multiple Births in Europe: Risk and Pregnancy Outcomes

Boyle B

The increase in multiple births acrossEuropeis due to the increasing use of assisted reproductive therapies (ART), and to increasing maternal age. Where foetuses from multiple pregnancies are affected by congenital anomaly; this may affect prenatal diagnosis and outcome. This paper aims to assess risk of congenital anomaly in multiple births, and differences in prenatal diagnosis and pregnancy outcome in affected multiple pregnancies.

Data from EUROCAT population based congenital anomaly registries in 14 European countries, representing over 3 .3 million births 2000-2007 were analysed, including 91,000 congenital anomaly cases Registry specific population births data for singleton and multiple deliveries were also obtained.

The population multiple birth rate was approximately 3%. The relative risk of non-chromosomal congenital anomaly in multiple births relative to singletons was 1.39 (95%CI 1.35-1.44), and for chromosomal anomalies 0.65 (0.56-0.74). In approximately 10% of affected twin pairs, both babies are malformed, but not necessarily the same malformation. While congenital anomalies in multiple births are as likely to be diagnosed prenatally, they are less likely than singletons to result in termination, but more likely to result in stillbirth. While multiple births are slightly more likely to be live born there is no difference in early neonatal death rates.

The excess risk of non-chromosomal congenital anomaly in multiple births may result from risk associated to twinning as well as risk associated with ART. Policies of single embryo transfer should lead to lowering of the rate of multiple births, but may simply transfer ART-related congenital anomaly risk to singletons.